

WHAT IS CLAIMED IS:

1                    1.        A method of inhibiting expression of an endogenous cellular gene  
 2        in a cell, the method comprising the step of:  
 3                    contacting a first target site in the endogenous cellular gene with a first  
 4        zinc finger protein, wherein the  $K_d$  of the zinc finger protein is less than about 25 nM;  
 5                    thereby inhibiting expression of the endogenous cellular gene by, at least  
 6        about 20%.

1                    2.        The method of claim 1, wherein the step of contacting further  
 2        comprises contacting a second target site in the endogenous cellular gene with a second  
 3        zinc finger protein.

1                    3.        The method of claim 2, wherein the first and second target sites are  
 2        adjacent.

1                    4.        The method of claim 3, wherein the first and second zinc finger  
 2        proteins are covalently linked.

1                    5.        The method of claim 1, wherein the first zinc finger protein is a  
 2        fusion protein comprising a regulatory domain.

1                    6.        The method of claim 5, wherein the first zinc finger protein is a  
 2        fusion protein comprising at least two regulatory domains.

1                    7.        The method of claim 2, wherein the first and second zinc finger  
 2        proteins are fusion proteins, each comprising a regulatory domain.

1                    8.        The method of claim 7, wherein the first and second zinc finger  
 2        protein are fusion proteins, each comprising at least two regulatory domains.

1                    9.        A method of inhibiting expression of an endogenous cellular gene  
 2        in a cell, the method comprising the step of:  
 3                    contacting a target site in the endogenous cellular gene with a fusion zinc  
 4        finger protein comprising six fingers and a regulatory domain, wherein the  $K_d$  of the zinc  
 5        finger protein is less than about 25 nM;

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1                    10.    The method of claim 1, wherein the cell is selected from the group  
2    consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

12. The method of claim 11, wherein the cell is a human cell.

1 ~~subpg~~ 14. The method of claim 1, wherein the endogenous cellular gene is a  
2 selected from the group consisting of VEGF, ER $\alpha$ , IGF-I, c-myc, c-myb, ICAM, and  
3 Her2/Neu.

1                    16.    The method of claim 1, wherein the inhibition of gene expression  
2 prevents gene activation.

1 18. The method of claim 1, wherein the method further comprises the  
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,  
3 wherein the delivery vehicle comprises a liposome or a membrane translocation  
4 polypeptide.

1                    19.    The method of claim 1, wherein the zinc finger protein is encoded  
2    by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the  
3    method further comprises the step of first administering the nucleic acid to the cell in a  
4    lipid:nucleic acid complex or as naked nucleic acid.

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~~of claim~~

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1 31. A method of activating expression of an endogenous cellular gene,  
 2 the method comprising the step of:  
 3 contacting a first target site in the endogenous cellular gene with a first  
 4 zinc finger protein, wherein the  $K_d$  of the zinc finger protein is less than about 25 nM;  
 5 thereby activating expression of the endogenous cellular gene to at least  
 6 about 150%.

1 32. The method of claim 31, wherein the step of contacting further-  
 2 comprises contacting a second target site in the endogenous cellular gene with a second  
 3 zinc finger protein.

1 33. The method of claim 32, wherein the first and second target sites  
 2 are adjacent.

1 34. The method of claim 33, wherein the first and second zinc finger  
 2 proteins are covalently linked.

1 35. The method of claim 31, wherein the first zinc finger protein is a  
 2 fusion protein comprising a regulatory domain.

1 36. The method of claim 35, wherein the first zinc finger protein is a  
 2 fusion protein comprising at least two regulatory domains.

1 37. The method of claim 32, wherein the first and second zinc finger  
 2 proteins are fusion proteins, each comprising a regulatory domain.

1 38. The method of claim 37, wherein the first and the second zinc  
 2 finger protein are fusion proteins, each comprising at least two regulatory domains.

1 39. A method of activating expression of an endogenous cellular gene,  
 2 the method comprising the step of:  
 3 contacting a target site in the endogenous cellular gene with a fusion zinc  
 4 finger protein comprising six fingers and a regulatory domain, wherein the  $K_d$  of the zinc  
 5 finger protein is less than about 25 nM;  
 6 thereby activating expression of the endogenous cellular gene to at least  
 7 about 150%.

1 40. The method of claim 31, wherein the cell is selected from the  
2 group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, or a  
3 fungal cell.

1 41. The method of claim 40, wherein the cell is a mammalian cell.

1 42. The method of claim 41, wherein the cell is a human cell

1 43. The method of claim 31, wherein expression of the endogenous  
2 cellular gene is activated to at least about 200-500%.

1 ~~44. The method of claim 31, wherein the endogenous cellular gene is a~~  
2 ~~selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-~~  
3 ~~R.~~

1 45. The method of claim 31, wherein the endogenous cellular gene is  
2 VEGF.

1 46. The method of claim 31, wherein the activation of gene expression  
2 prevents repression of gene expression.

1 47. The method of claim 35 or 37, wherein the regulatory domain is  
2 selected from the group consisting of a transcriptional activator, or a histone  
3 acetyltransferase.

1 ~~48. The method of claim 31, wherein the method further comprises the~~  
2 ~~step of first administering to the cell a delivery vehicle comprising the zinc finger protein,~~  
3 ~~wherein the delivery vehicle comprises a liposome or a membrane translocation~~  
4 ~~polypeptide.~~

1 49. The method of claim 31, wherein the zinc finger protein is encoded  
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the  
3 method further comprises the step of first administering the nucleic acid to the cell in a  
4 lipid:nucleic acid complex or as naked nucleic acid.

1 50. The method of claim 31, wherein the zinc finger protein is encoded  
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a

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3 promoter, and wherein the method further comprises the step of first administering the  
4 expression vector to the cell.

1 51. The method of claim 50, wherein the expression vector is a viral  
2 expression vector.

1 52. The method of claim 50, wherein the expression vector is a  
2 retroviral expression vector, an adenoviral vector, a DNA plasmid vector, or an AAV  
3 expression vector.

1 53. The method of claim 50, wherein the zinc finger protein is encoded  
2 by a nucleic acid operably linked to an inducible promoter.

1 54. The method of claim 50, wherein the zinc finger protein is encoded  
2 by a nucleic acid operably linked to a weak promoter.

1 55. The method of claim 31, wherein the cell comprises less than about  
2  $1.5 \times 10^6$  copies of the zinc finger protein.

1 56. The method of claim 31, wherein the target site is upstream of a  
2 transcription initiation site of the endogenous cellular gene.

1 57. The method of claim 31, wherein the target site is adjacent to a  
2 transcription initiation site of the endogenous cellular gene.

1 58. The method of claim 31, wherein the target site is adjacent to an  
2 RNA polymerase pause site downstream of a transcription initiation site of the  
3 endogenous cellular gene.

1 59. The method of claim 31, wherein the zinc finger protein comprises  
2 an SP-1 backbone.

1 60. The method of claim 59, wherein the zinc finger protein comprises  
2 a regulatory domain and is humanized.

1 61. A method of modulating expression of an endogenous cellular gene  
2 in a cell, the method comprising the step of:

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71. The method of claim 70, wherein the cell is a mammalian cell

72. The method of claim 71, wherein the cell is a human cell.

73. ~~The method of claim 61, wherein the endogenous cellular gene is a selected from the group consisting of VEGF, ER $\alpha$ , IGF-I, c-myc, c-myb, ICAM, Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.~~

74. The method of claim 61, wherein the endogenous cellular gene is VEGF.

75. The method of claim 65 or 67, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, a transcriptional activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone deacetylase.

76. ~~The method of claim 61, wherein the method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide.~~

77. ~~The method of claim 61, wherein the zinc finger protein is encoded by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the nucleic acid to the cell in a lipid:nucleic acid complex or as naked nucleic acid.~~

78. The method of claim 61, wherein the zinc finger protein is encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the expression vector to the cell.

79. ~~The method of claim 78, wherein the expression vector is a viral expression vector.~~

80. ~~The method of claim 78, wherein the expression vector is a retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression vector, or an AAV expression vector.~~



1                    82.     The method of claim 78, wherein the zinc finger protein is encoded  
2     by a nucleic acid operably linked to a weak promoter.

1                    83.     The method of claim 61, wherein the cell comprises less than about  
2      $1.5 \times 10^6$  copies of the zinc finger protein.

1                    84.     The method of claim 61, wherein the target site is upstream of a  
2     transcription initiation site of the endogenous cellular gene.

1           85.     The method of claim 61, wherein the target site is adjacent to a  
2     transcription initiation site of the endogenous cellular gene.

1 86. The method of claim 61, wherein the target site is adjacent to an  
2 RNA polymerase pause site downstream of a transcription initiation site of the  
3 endogenous cellular gene.

1                    87.     The method of claim 61, wherein the zinc finger protein comprises  
2     an SP-1 backbone.

1            88.     The method of claim 88, wherein the zinc finger protein comprises  
2     a regulatory domain and is humanized.